Clostridium difficile in Poultry and Poultry Meat

Roger B. Harvey,[1] Keri N. Norman,[2] Kathleen Andrews,[1] Michael E. Hume,[1] Charles M. Scanlan,[3]
Todd R. Callaway,[1] Robin C. Anderson,[1] and David J. Nisbet[1]

Abstract

The incidence and severity of disease associated with toxigenic *Clostridium difficile* have increased in hospitals in North America from the emergence of newer, more virulent strains. Toxigenic *C. difficile* has been isolated from food animals and retail meat with potential implications of transfer to human beings. The objective of the present study was to determine the prevalence of toxigenic *C. difficile* in chickens and retail poultry meat in Texas. Seven *C. difficile* isolates were detected in fecal samples of 300 (2.3%) broiler chickens. Three cultivation procedures were evaluated for isolation of *C. difficile* from poultry meat and detected 1/32 (3.1%), 2/32 (6.2%), and 4/32 (12.5%) for the three procedures, respectively. Chicken and poultry meat isolates were characterized as toxinotype V and pulsed-field gel electrophoresis gel type-NAP7 or NAP7-variant. Susceptibilities to 11 antimicrobial agents in the current study suggested somewhat reduced resistance than reported for other meat or animal toxinotype V isolates.

SINCE 2003, THE INCIDENCE and severity of disease associated with toxigenic *Clostridium difficile* have increased in hospitals in North America. Indications are that these increases may be due to the emergence of a new strain of toxigenic *C. difficile* (restriction endonuclease analysis type BI, North American pulsed-field gel electrophoresis [PFGE] pattern 1 [BI/NAP1], toxinotype III) that exhibits increased levels of resistance, virulence, and toxin production (McDonald *et al.*, 2005). Various strains of *C. difficile*, including NAP1, toxinotype III, can be isolated from food animals and meat (Songer *et al.*, 2009; Weese *et al.*, 2010); however, the predominant strains from food animals are NAP7 and NAP8, toxinotype V (Jhung *et al.*, 2008). Because food animals can be colonized by *C. difficile*, and the bacterium has been isolated from retail meats and poultry, some researchers speculate that *C. difficile* is a food-associated organism and consumption of contaminated meat could be responsible for increased community-associated *C. difficile* infection (Jhung *et al.*, 2008; Songer *et al.*, 2009). The objective of the present study was to determine the prevalence of toxigenic *C. difficile* in commercial poultry and poultry meat in Texas.

In 2009, the authors collected fecal samples from 42-day-old broiler chickens (*n*=300, 50 each from six separate barns). Alcohol shock, enhanced enrichment and/or concentration techniques, selective media, and anaerobic incubation were utilized as described for procedures to cultivate *C. difficile* from fecal samples (Norman *et al.*, 2009).

In July 2010, the authors visited five grocery meat markets in Bryan and College Station, TX, and collected packages of in-house store brands and nationally recognized brands of poultry wings, thighs, legs, and breasts. A total of 32 meat samples were cultivated for *C. difficile*.

When cultivating meat samples for the procedures below, inocula were obtained by placing two pieces (leg, wing, or thigh) or one breast into a sterile 530-mL plastic bag to which 50 mL of phosphate-buffered saline (PBS) was added, the bag sealed, and massaged by hand for 1 min.

Procedure A: This procedure has been described previously for poultry meat (Weese *et al.*, 2010) and uses a 48 h enrichment time.

Procedure B: This procedure has been described previously for retail meat (Rodriguez-Palacios *et al.*, 2007; Harvey *et al.*, 2011) and utilizes 14 days of enrichment time.

Procedure C: This procedure is a modification of Procedure B in that volumes of wash and enrichment broth were both increased. Ten milliliters of the PBS wash was added to 20 mL of enrichment broth, incubated anaerobically at 37°C for 14 days, streaked onto cycloserine cefoxitin fructose agar (CCFA), Brucella, and CCFA-HT agar (Anaerobe Systems, Walnut Hill, CA), and incubated anaerobically at 37°C for 5 days.

Presumptive diagnosis in all procedures consisted of the presence of colonies morphologically similar to *C. difficile*, L-proline aminopeptidase activity (Pro Disc; Remel Inc., Lenexa, KS), biochemical characterization, and by the presence of the *tcdC* regulatory gene.

[1]Food and Feed Safety Research Unit, SPARC, Agricultural Research Service, U.S. Department of Agriculture, College Station, Texas.
[2]Agricultural Research Service, U.S. Department of Agriculture, U.S. Meat Animal Research Center, Clay Center, Nebraska.
[3]Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas.

TABLE 1. ANTIMICROBIAL INTERPRETIVE CATEGORIES AND INTERPRETIVE RESULTS OF *CLOSTRIDIUM DIFFICILE*
FROM SEVEN CHICKEN FECAL ISOLATES AND SEVEN POULTRY MEAT ISOLATES IN TEXAS[a]

Antimicrobial	Sensitive[b]		Intermediate		Resistant	
	Fecal	Meat	Fecal	Meat	Fecal	Meat
Amoxicillin-clavulanic acid	7S (100)	7S (100)				
Ampicillin			7I (100)	7I (100)		
Cefoxitin					7R (100)	7R (100)
Chloramphenicol	7S (100)	7S (100)				
Ciprofloxacin			2I (28)		5R (72)	7R (100)
Clindamycin	3S (43)		4I (57)	2I (28)		5R (72)
Imipenem	6S (86)				1R (14)	7R (100)
Metronidazole	7S (100)	7S (100)				
Piperacillin-tazobactam	7S (100)	7S (100)				
Tetracycline	7S (100)	6S (86)				1R (14)
Vancomycin	7S (100)	7S (100)				

[a]Results interpreted according to the Clinical and Laboratory Standards Institute: S, sensitive; I, intermediate; R, resistant; ciprofloxacin and vancomycin interpretation based on values for trovafloxacin and Gram-positive aerobes, respectively (CLSI, 2007).
[b]Numbers in parentheses are percentages.

PCR was used to detect toxin A (*tcdA*) and B (*tcdB*) genes, *tcdC* gene deletion, toxinotyping, and *cdtB* binary toxin gene and have been described (Killgore *et al.*, 2008; Harvey *et al.*, 2011). Each *C. difficile* isolate was tested for susceptibility to 11 antimicrobial agents (Table 1) by use of a commercially available test (Etest; AB Biodisk North America Inc., Piscataway, NJ) according to the manufacturer's recommendations and have been described (Norman *et al.*, 2009; Harvey *et al.*, 2011).

PFGE was used to compare genotypes of *C. difficile* isolates. The PFGE procedures followed techniques of a modified 7-day protocol utilized by the CDC (A. Thompson, personal communication, 2008) and have been described (Killgore *et al.*, 2008; Harvey *et al.*, 2011).

Seven of 300 (2.3%) samples from the market age broilers were positive for *C. difficile* and were toxinotype V and PFGE North America Pulsed-Field type (NAP) 7-variant (91% similar to NAP7). This is the first report of toxinotype V, PFGE NAP7-variant isolated from chickens. The low isolation rate of the present study is in sharp contrast to a Slovenian study which reported a prevalence of 41%–100% (toxinotype 0 and IV) in Leghorn pullets (Zidaric *et al.*, 2008) and a Zimbabwe study in which broiler chickens had a prevalence of 29% (Simango and Mwakurudza, 2008). However, a prevalence of 5% in broiler chickens in Austria compares favorably with the results of this study (Indra *et al.*, 2009). Toxinotype V, PFGE types NAP7 or NAP8 are the most commonly reported *C. difficile* strains in food animals (Jhung *et al.*, 2008); however, up until now, there have been no reports of these strains found in poultry.

Seven of the 32 poultry meat samples were positive utilizing the three different isolation procedures (7/96 = 7.3%). When cultivation techniques were compared, Procedure A detected 1/32 (3.1%), B detected 2/32 (6.3%), and C detected 4/32 (12.5%). One sample produced two different isolates utilizing Procedures A and C. All seven isolates were toxinotype V, three were NAP7 and four were NAP7-variant. To the authors' knowledge, this is the first report for toxinotype V and PFGE NAP7 in poultry meat. A *C. difficile* prevalence of 12.5% in poultry meat of the present study is very similar to a

12.8% prevalence (ribotype 078) reported for poultry meat (Weese *et al.*, 2010).

Antimicrobial susceptibilities of *C. difficile* meat isolates in the current study (Table 1) are similar to toxinotype V, ribotype 078 isolates reported for meat and pigs (Jhung *et al.*, 2008; Norman *et al.*, 2009; Songer *et al.*, 2009; Harvey *et al.*, 2011); however, the poultry fecal isolates appear to have less resistance than the poultry meat isolates of the current study.

Limitations of the present study would include the natural bias of cultivation to determine prevalence, the small sample size, and the limited geographical area for sample collection. The authors are unsure of the clinical relevance of isolation of *C. difficile* from chickens and poultry meat as pertains to potential transfer of *C. difficile* from poultry to meat and humans.

Disclosure Statement

No competing financial interests exist.

Disclaimer

Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, and/or exclusion of others that may be suitable.

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Address correspondence to:
Roger B. Harvey, D.V.M., M.S.
Food and Feed Safety Research Unit
SPARC
Agricultural Research Service
U.S. Department of Agriculture
2881 F&B Road
College Station, TX 77845

E-mail: roger.harvey@ars.usda.gov